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Checkpoint Mediators: Relaying Signals from DNA Strand Breaks

Dispatch

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The recently identified checkpoint mediator MDC1 facilitates recruitment of DNA repair proteins to damaged sites and establishment of the intra-S-phase cell-cycle checkpoint. Increasing evidence suggests that proteins like MDC1 provide the framework necessary for transducing signals from DNA double-strand breaks.

A swift and accurate cellular response to DNA damage is crucial in preventing genomic instability. Within minutes, a collection of DNA damage response proteins rapidly localize to sites of double-strand breaks, forming characteristic immunofluorescent foci which are believed to have some function in DNA repair. Recent work in several laboratories has led to the discovery of yet another protein that migrates to DNA strand breaks, the Mediator of DNA Damage Checkpoint protein 1 (MDC1) protein. MDC1 is the third member in the growing family of checkpoint proteins known as 'mediators', the other two being the tumor suppressor gene product BRCT1 and the p53-binding protein 53BP1. These nuclear factors all contain BRCT protein–protein interaction motifs and are necessary for transduction of DNA damage signals to the checkpoint apparatus by the kinases ATM and ATR (which are related to each other and to phosphatidylinositide 3-kinase). MDC1 is one of the earliest proteins to localize at DNA double-strand breaks and appears to be necessary for linking ATM to several of its effectors that rapidly downregulate DNA synthesis in response to ionizing radiation [1–5].

In response to double-strand breaks, ATM rapidly phosphorylates a collection of proteins that regulate transitions through the cell cycle and/or induce apoptosis [6]. Cells derived from ataxia telangiectasia (AT) patients, which lack ATM function, exhibit a characteristic radioresistant DNA synthesis phenotype which results from their inability to reduce initiation and elongation of DNA synthesis after ionizing radiation. This ATM-regulated pathway is also referred to as the 'intra-S-phase' checkpoint, distinguishing it from an entirely different pathway that prevents cells from undergoing mitosis when DNA replication is incomplete [7]. Several components of the intra-S-phase checkpoint pathway were discovered because mutations in their genes, like *ATM* mutations, result in genomic instability syndromes which share radioresistant DNA synthesis as a common cellular phenotype; for example, *Mre11* mutations cause an AT-like disorder, and *NBS1* mutations result in Nijmegen breakage syndrome [6]. Additional participants were later discovered as targets of the ATM kinase — these include BRCA1, 53BP1, FANCD2, SMC1 and NBS1 — and many pre-exist in a large complex ready to respond to DNA damage [6,8].

MDC1's potential involvement in the intra-S-phase checkpoint was suggested when it was discovered to associate with either the Mre11–NBS1–Rad50 repair complex or the protein kinase Chk2, components of two separable pathways regulated by ATM that cooperate to inhibit DNA synthesis optimally in irradiated cells [1–3,9]. Chk2 is considered a major effector of ATM, acting to promote degradation of the phosphatase Cdc25A and thereby prevent activation of the cyclin-dependent kinase Cdk2 and initiation of DNA replication [9]. MDC1 associates with Chk2 after cells are exposed to ionizing radiation, an interaction mediated through the FHA domain of MDC1 and the phospho-threonine 68 region of Chk2, which is phosphorylated by ATM in response to ionizing radiation [3]. By associating with MDC1, Chk2 contributes to the rapid phosphorylation of MDC1 in irradiated cells [3]. But MDC1 does not appear to be necessary for ATM to phosphorylate Chk2 or for Chk2 to participate in the intra-S phase checkpoint. This is inferred from the unexpected observation that MDC1 does not seem to be necessary for degradation of Cdc25A in irradiated cells [2]. Instead, MDC1 facilitates phosphorylation of p53 by Chk2 and promotes apoptosis — a permanent solution to the problem of controlling genomic instability [3].

How does MDC1 assist the transduction of ATM-associated signals from DNA double-strand breaks to proteins such as NBS1, thereby promoting induction of the intra-S-phase checkpoint? Recent evidence suggests that ATM is expressed as one or more inactive dimers that undergo autophosphorylation when changes in chromatin structure occur as a result of DNA strand breaks [10]. Activated ATM rapidly phosphorylates the histone H2A variant H2AX, tagging DNA double-strand breaks and forming the initial signal for the subsequent migration of other checkpoint proteins to ionizing radiation-induced foci [11,12]. MDC1 is one of the earliest proteins that binds directly to phosphorylated H2AX (H2AX-γ) [1–5]. Furthermore, the localization of MDC1 with H2AX-γ is necessary for the sequential recruitment of 53BP1, BRCA1 and the Mre11–NBS1–Rad50 complex to the H2AX-γ–tagged sites, events that correlate with an intact intra-S phase checkpoint [1,2,11,13].

ATM-dependent phosphorylation of BRCA1, NBS1 and two additional players in the NBS1 branch of the pathway — the chromosomal structural maintenance protein SMC1, important for sister chromatid cohesion, and FANCD2, mutated in a subtype of the chromosome instability disorder Fanconi anemia — are also needed for an intact intra-S phase checkpoint [6,8]. Unexpectedly, hyperphosphorylation of most ATM target proteins, with the exception of BRCA1, is largely

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intact in MDC1-disrupted cells [1,2,14]. Therefore, the ability of MDC1 to directly bind to H2AX-γ and recruit members of the NBS1 pathway to sites of DNA damage may be one of its essential functions, providing a framework for ATM-regulated responses.

How might MDC1 and H2AX-γ convey signals to other checkpoint proteins? MDC1 and H2AX are both needed for the recruitment of 53BP1 to ionizing radiation-induced foci [1,14,15]. Like MDC1, 53BP1 also colocalizes early with H2AX-γ in irradiated cells [16]. In turn, 53BP1 is necessary for both optimal phosphorylation of BRCA1 by ATM and recruitment of BRCA1 to ionizing radiation-induced foci, events that correlate with phosphorylation of the Chk1 kinase and optimal execution of the G2 checkpoint as well as the intra-S phase checkpoint [14–16]. Not all functions of 53BP1 and MDC1 overlap, however, as elimination of 53BP1 expression seems to have more adverse effects on ATM's connection to downstream targets such as BRCA1, SMC1 and Chk2 [16–18]. Furthermore, loss of 53BP1 function does not interfere with NBS1 foci formation, whereas loss of MDC1 function does [16]. MDC1 and H2AX thus appear to sit at the top of the checkpoint hierarchy, with ATM at the helm, and together, MDC1 and 53BP1 link ATM to many of its effectors (Figure 1).

It will be interesting to see how the components of this complicated network cooperate to promote DNA repair and execute cell-cycle checkpoints, such as that mediated by the NBS1/SMC1 pathway where it is mostly unclear how the proteins regulate DNA replication. This will involve finding other endpoints besides focus formation to assess integrity of biological function. Regardless, enormous progress has been made in recent years in identifying and characterizing gene products important for preserving genomic stability and cell viability as demonstrated in the work discussed here.

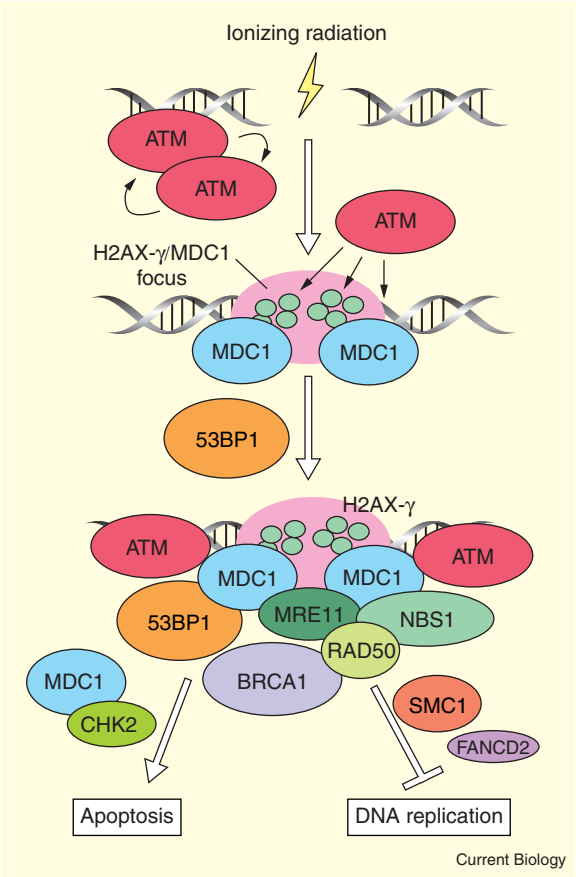

Figure 1. A model for the checkpoint function of MDC1 in irradiated cells.

Throughout nuclear chromatin, inactive ATM dimers respond to DNA double-strand breaks by undergoing autophosphorylation and activation. Activated ATM molecules migrate to sites of damage and phosphorylate histone H2AX, forming H2AX-γ foci. MDC1 directly associates with H2AX-γ and subsequently recruits 53BP1 and BRCA1, followed by the Mre11–NBS1–Rad50 repair complex. MDC1-associated mobilization of the Mre11–NBS1–Rad50 complex is necessary for efficient induction of the intra-S-phase checkpoint in cooperation of two additional ATM targets, the chromatin structure protein, SMC1 and the Fanconi anemia protein FANC2. How these proteins interact to inhibit DNA replication is still unknown. MDC1 also binds to the Chk2 protein kinase and stimulates its ability to promote apoptosis.

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